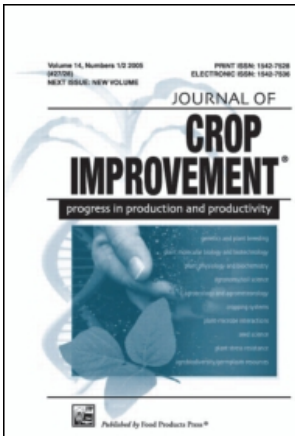

Journal of Crop Improvement
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t792303981

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Online publication date: 06 November 2010

To cite this Article Van Nurden, A. , Scott, R. A. , Hesler, L. , Tilmon, K. , Glover, K. and Carter, C.(2010) 'Inheritance of Soybean Aphid Resistance from PI 71506', Journal of Crop Improvement, 24: 4, 400 — 416
To link to this Article: DOI: 10.1080/15427528.2010.511104
URL: http://dx.doi.org/10.1080/15427528.2010.511104

Journal of Crop Improvement, 24:400–416, 2010

ISSN: 1542-7528 print/1542-7535 online
DOI: 10.1080/15427528.2010.511104

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Inheritance of Soybean Aphid Resistance from PI 71506

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Resistance to the soybean aphid (Aphis glycines Matsumura) was characterized in segregating populations from crosses of soybean [Glycine max (L.) Merr.] accession PI 71506 to susceptible cultivars, and compared to Rag1 resistance from the cultivar 'Dowling.' Two susceptible adapted cultivars were crossed with PI 71506 or Dowling. In no-choice greenhouse assays, resistance corresponded to a single dominant gene model for both the PI 71506-derived and Dowling-derived populations. Segregation of aphid resistance in SD1111RR × PI 71506 $F_{2:3}$ populations in aphid field-cage trials also fit a single-gene model, as did segregation of aphid resistance in the $F_{2:5}$ generation. However, other genetic effects may also contribute to aphid resistance from PI 71506. Comparison with Rag1 resistance from Dowling indicated that PI 71506 resistance was weaker than that associated with Rag1, but antixenosis resistance from PI 71506 was effective against an Ohio aphid biotype that has overcome Rag1 resistance.

KEYWORDS *Soybean aphid,* Aphis glycines*, PI 71506, host-plant resistance*

This research was supported in part by the South Dakota Soybean Research and Promotion Council.

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INTRODUCTION

The soybean aphid (*Aphis glycines* Matsumura), first discovered in the United States in the summer of 2000, has spread across much of the nation's soybean-production areas (Ragsdale, Voegtlin, & O'Neil 2004) and has quickly become an insect pest of economic importance to soybean producers. Large aphid populations cause damage directly by reducing soybean plant seed size, pods per plant, and seeds per pod, resulting in substantial effects on yield potential (Beckendorf, Catangui, & Riedell 2008). Only three years following its introduction into the United States, the soybean aphid had already caused 16 bushels per acre yield losses in Iowa soybean fields (Rice, O'Neal, & Pedersen 2005). A survey of Iowa soybean growers estimated economic losses of one quarter of a billion dollars from soybean aphids in 2003 (Pilcher, Rice, & Vagts 2005). In addition to the economic impact of yield loss caused by aphid feeding damage, soybean aphids are capable of transmitting soybean mosaic virus (Wang & Ghabrial 2002), which can cause drastic yield loss and is spread easily when infected seed serves as inoculum (Hill et al. 1987). The economic risk of soybean aphid infestations and the extent of their establishment in North America make it imperative to identify and exploit new sources of genetic resistance to the soybean aphid in the development of aphid-resistant soybean varieties.

Host-plant resistance is a sustainable and environmentally safe way to limit damage from soybean aphids. Antibiosis, antixenosis, and tolerance are three types of insect resistance exhibited by plants (Painter 1951; Smith 2005). Non-preference or antixenosis resistance suggests the presence of morphological or chemical plant factors that influence aphid preferences when selecting host plants (Smith 2005). In contrast, antibiosis affects the survival and reproduction of aphids when feeding on resistant plants (Smith 2005). In comparison with non-preference, antibiosis-based resistance is considered more reliable because it reduces aphid reproduction and survival (Mebrahtu, Kraemer, & Andebrhan 2002), making it more desirable to soybean breeders. However, components that lead to an antibiotic effect in a no-choice environment may also act as a deterrent to the predator when it has a choice of feeding sites, and conversely antixenosis can also lead to effects on survival and reproduction (Diaz-Montano et al. 2007). Thus, in practice, differentiating between antibiosis and antixenosis is difficult (Smith 2005).

After the discovery of soybean aphids in the United States, many accessions from the USDA soybean germplasm collection were screened for resistance (Hill, Li, & Hartman 2004; Mensah et al. 2005). Dowling, Jackson, PI 71506, PI 230977, Sugao Zarai, Sato, and T260H were identified as resistant by Hill, Li, and Hartman (2004). Dowling and Jackson were shown to have antibiosis resistance, whereas PI 71506 showed antixenosis resistance (Hill, Li, & Hartman 2004). Mensah et al. (2005) identified antibiosis resistance in the early-maturing accessions PI 567541B and PI 567598B, and

antixenosis in PI 567543C and PI 567597C. Mian, Hammond, and St. Martin (2008) identified aphid resistance in several accessions, including PI 243540, which displayed strong antibiosis resistance. Hesler, Dashiell, and Lundgren (2007) further characterized resistance in various soybean lines, confirming both antixenosis and antibiosis qualities in PI 71506 and PI 230977.

Genetic analysis of antibiosis resistance in Dowling and Jackson has revealed inheritance controlled by a single dominant gene, *Rag1* (Hill, Li, & Hartman 2006), which was mapped to linkage group M/7 by Li et al. (2007). Shortly after the discovery of *Rag1*, aphid biotypes were identified that could overcome its resistance (Kim et al. 2008). One of these, the Ohio aphid biotype, was discovered and collected from Ohio soybean fields. This discovery prompted a search for new resistance genes. A second gene, *Rag2,* in soybean accession PI 243540 was identified as a single dominant gene that provided antibiosis resistance to the Ohio aphid biotype (Kang, Mian, & Hammond 2008; Mian, Hammond, & St. Martin 2008). The *Rag2* gene was later mapped to a locus on linkage group F, confirming it as a new source of resistance (Mian et al. 2008). Two other soybean lines, PI 200538 and PI 567597C, have also been identified as possessing resistance to the Ohio aphid biotype (Kim et al. 2008).

Soybean aphid resistance in PI 567541B and PI 567598B is inherited as two recessive genes (Mensah, DiFonzo, & Wang 2008) or QTL (Zhang, Gu, & Wang 2008). The QTL for aphid resistance from PI 567541B were confirmed and mapped to linkage groups M and F (Zhang, Gu, & Wang 2008). The QTL on M had a larger effect and corresponded with the *Rag1* region, whereas the QTL on F had a smaller effect and did not correspond closely with any other identified aphid-resistance genes (Zhang, Gu, & Wang 2008). Aphid screening revealed that PI 567541B was also resistant to the Ohio isolate (Kim et al. 2008).

Although several other soybean accessions with aphid resistance have been identified, little is known about their genetic makeup. Furthermore, the mechanisms of resistance exhibited are not always easily discernable. PI 71506 could be a useful source of resistance because previous work (Hesler, Dashiell, & Lundgren 2007) indicated that it could contain genes for both antibiosis and antixenosis. The objectives of this investigation were, therefore, to characterize the soybean-aphid resistance from PI 71506, determine its mode of inheritance, and compare the aphid resistance from PI 71506 with that of *Rag1* from Dowling.

MATERIALS AND METHODS

Plant Material

Crosses were made between released South Dakota State University variety SD1111RR and PI 71506 (as pollen parent) in summer 2006. A similar

cross of experimental line SD01-3219R and PI 71506 (as pollen parent) was also made. Both SD parents were soybean-aphid susceptible, group I maturity soybeans that have purple flowers and gray pubescence. The soybean aphid-resistant parent, PI 71506, introduced from China in cooperation with C.M. Hehm in 1927, is a maturity group IV soybean accession with purple flowers and gray pubescence. To create similar segregating populations for comparison, SD1111RR and SD01-3219R were also crossed with Dowling (as pollen parent), a maturity group VIII soybean aphid-resistant accession containing the known soybean aphid resistance gene *Rag1*. Crosses were harvested, and resulting F_1 seeds were grown in the greenhouse in fall of 2006 to spring of 2007. F_1 plants of each cross were threshed individually to obtain F_2 seeds, which were planted in the greenhouse in March 2007 to produce segregating F_2 populations for phenotypic evaluation. After evaluation, the F_2 plants were harvested and threshed individually. The resulting seed was used to create segregating $F_{2:3}$ populations, which were planted in field cage trials in summer of 2008. The $F_{2:3}$ plants of the SD1111RR × PI71506 population in the field cage were harvested individually in fall 2008, and the $F_{2:4}$ generation was grown in the greenhouse during the winter of 2008–09. Seeds were harvested in March 2009 and the $F_{2:5}$ populations were planted in the field in 2009.

Soybean-Aphid Resistance Screening of F_2 Populations

All F_2 populations, parents, and check varieties were planted in a single greenhouse trial in March 2007. Seeds were planted at a rate of one seed per pot in 4-in. square plastic pots containing one part sand, one part #1 Sunshine potting mix (Sun Gro Horticulture Ltd., Canada), and one part field soil. Along with the segregating populations, three replicates of susceptible and resistant checks were planted. Resistant parent PI 71506, susceptible parents SD1111RR and SD01-3219R, and LD(05)16137, a line containing confirmed *Rag1* resistance derived from Dowling, were used as checks. When a majority of the plants reached the V2 growth stage, small sticky cages (Diaz-Montano et al. 2006) were attached to the top of the first trifoliate leaf at a rate of one per plant. A single adult aphid was placed in each cage and the cages were sealed. The soybean aphids were procured from colonies maintained at the USDA-ARS North Central Agricultural Research Laboratory and represented samples taken from Brookings County, SD, USA. Aphids were given a period of one week in the cages to feed and develop viviparous offspring. Cages were monitored and resealed as needed throughout the trial. After seven days, the number of nymphs produced and adult aphid-mortality data were collected from each plant. Upon completion of the study, an insecticide treatment was used to kill any remaining aphids and plants were allowed to grow to full maturity.

Soybean Aphid Resistance Screening of $F_{2:3}$ and $F_{2:5}$ Populations

In the spring of 2008, $F_{2:3}$ populations were planted in a 19.5 m × 12.8 m field cage located in Aurora, SD (44° 17' N, 96° 41' W). Up to 15 seeds from each $F_{2:3}$ family were planted in 0.9 m rows. Plots in the field cage were arranged in three blocks containing 22 3.7-m rows that were spaced 76 cm and 38 cm alternately. Each 3.7 m row contained four 0.9 m $F_{2:3}$ population rows planted in series. SD1111RR, PI71506, and Dowling, as well as 'S19-R5,' a susceptible Syngenta cultivar, were also planted in 0.9 m rows as checks. Each check had three replicates that were planted among the populations in the cage. Each plant in the cage was infested with 5 adult aphids per plant on July 14, 2008. The soybean-aphid cultures used to infest the cage were collected from area soybean fields and reared in small field cages. On the day of infestation, leaf samples containing several hundred soybean aphids were harvested from the rearing cages, placed in plastic bags with a moist paper towel and transported in a cooler to the aphid cage. Aphids were placed on the undersides of the newest trifoliate of each plant in every row, using a fine-textured artist's brush. Plants were monitored until aphid populations reached threshold levels of 200 to 250 aphids per plant on susceptible check rows. Aphid threshold levels and differing resistance levels were achieved in two weeks and aphid ratings were recorded. Five plants in each $F_{2:3}$ row were given aphid ratings based on their estimated total aphid count, where 1 = 0–10 aphids; 2 = 11–100; 3 = 101–250; 4 = 251–500; 5 = 501–1000; and 6 = over 1,000 aphids. Estimation was achieved by counting up from the base of the plant until an aphid count of approximately 75 to 100 was reached. From this point on, the remaining aphids were estimated in increments of 10 to 25 until a total aphid count for the entire plant was obtained and an approximate aphid rating could be assigned.

The SD1111RR × PI71506 $F_{2:5}$ population was planted in the field at Aurora, SD, in May 2009. Natural aphid infestations were allowed to accumulate until susceptible check rows had ratings of 4 to 5, and the $F_{2:5}$ rows were rated for soybean-aphid resistance on a modified scale of 1 = ≤10 aphids; 2 = 10–49; 3 = 50–299; 4 = 300–499; 5 = 500–749; and 6 = ≥750 aphids, to give slightly higher stringency for assessing resistance and to discern incomplete dominance effects.

Ohio Aphid-Biotype Screening

We screened PI 71506 and SD1111RR against a soybean-aphid isolate from Ohio that has overcome *Rag1* resistance in Ohio and Illinois (Kim et al. 2008). A greenhouse performance test was planted in medium-sized, round plastic pots containing a mixture of one part sand, one part #1 Sunshine potting mix (Sun Gro Horticulture Ltd., Canada) and one part field soil.

Two seeds each of PI 71506, SD1111RR, and susceptible Syngenta variety S19-R5 were planted around the perimeter of each pot. Labeled stakes were placed next to the planted seeds for identification. Plants were thinned to one plant per line in each pot. Six replicates (pots) with this configuration were tested. When the soybean seedlings reached the V2 leaf stage, they were infested with 5 aphids per plant, and each pot was enclosed with a screened cover attached to the pots with tape. Pots were randomly placed on greenhouse benches. After one week, the number of adults and nymphs present on each plant were recorded. The total number of aphids (adults and nymphs) was calculated for each check variety within each replication to compare aphid count means. Following the test, all plant materials were frozen for several days to destroy any remaining aphids.

Data Analysis

In the greenhouse trial, F_2 plants were scored as resistant or susceptible based on nymph production during seven days. Resistant and susceptible levels were based on aphid reproduction and ratings observed on the resistant and susceptible check plants and rows. Plants were considered resistant if no nymphs were produced and susceptible if one or more nymphs were produced during the seven-day test-period. Observed resistant and susceptible individuals were compared to an expected single-dominant gene segregation ratio of 3:1. Chi-square values were calculated for each cross as well as the entire population. To compare check varieties in the F_2 greenhouse screening trial, total nymph production counts were collected from each plant for each variety. Nymph productions from 15 plants of each check variety were collected. These data were then analyzed with one-way fixed-effect ANOVA and a Student's *t*-test was used to find significant differences between specific variety means using JMP Version 4.0 (SAS Institute, 2002).

In the $F_{2:3}$ field-cage trial, aphid population ratings were taken from five plants within each check variety row. Fifteen plants were rated for each susceptible check and nine plants were rated from PI 71506. $F_{2:3}$ rows were scored as homozygous resistant, homozygous susceptible, or heterogeneous based on five individual plant ratings obtained from each row. Rows were scored homozygous resistant if all plant ratings were 2 or below, or as homozygous susceptible if all plant ratings were 3 or higher. Rows that contained both resistant (2 and below) and susceptible (3 and above) plants were scored as heterogeneous. Observed resistant, susceptible, and heterogeneous rows were compared to an expected single-gene segregation ratio of 1:2:1. Chi square values were calculated for each $F_{2:3}$ family as well as for the entire population using Microsoft Excel's Chi-square function. Analysis of variance was conducted on the check variety aphid-population rating means and Student's *t*-test was used to compare rating means between pairs of check varieties, by JMP Version 4.0 (SAS Institute, 2000).

In the 2009 field trial, aphid resistance in the naturally infested $F_{2:5}$ population of SD1111RR × PI 71506 was evaluated by visual observation and rating of each row using the modified rating scale (above) with higher stringency for resistance, where <50 aphids = ratings of 2 or less = resistant; 50–299 aphids = rating of 3 = intermediate; and 300 aphids or more = ratings of 4 and above = susceptible. Segregation of aphid resistance was analyzed by Chi-square test, and $F_{2:5}$ row aphid ratings were also analyzed by regression on the aphid rating of each $F_{2:3}$ plant from which the $F_{2:5}$s were derived by general linear model analysis of variance (SAS Institute, 2002).

Response to the Ohio-aphid isolate was analyzed with a one-way ANOVA using JMP Version 4.0 (SAS Institute, 2000) of soybean aphid counts from check varieties with a fixed-effects model. Differences between aphid count means for these check varieties were determined using the Student's *t*-test at the 0.05 probability level.

RESULTS

Segregation for nymph production in PI 71506-derived populations fit a single dominant-gene model in the no-choice greenhouse screening of F_2 populations (Table 1). The F_2 populations from the SD1111RR × Dowling cross also fit a 3:1 segregation ratio (Table 1), thus confirming the model of a single dominant gene. Three of five populations from SD01-3219R × Dowling also fit the single dominant-gene model (Table 1).

Population distributions for nymph production were skewed toward lower levels of nymph production in both the SD1111R × PI 71506 F_2 and the SD1111RR × Dowling F_2 populations (Figure 1), as well as in the SD01-3219R × PI71506 F2 and the SD01-3219R × Dowling F_2 populations (Figure 2). Nymph production differed among check varieties in the greenhouse screening trial (Figure 3), with significantly fewer nymphs produced on the *Rag1*-resistant LD05-16137 than on both susceptible checks. Significant differences were found between PI 71506 and SD01-3219R, but differences between PI 71506 and SD1111RR were not significant (Figure 3).

In the field cage screening, aphid ratings of the $F_{2:3}$ population rows from SD1111RR × PI 71506 segregated as expected for a single gene conditioning aphid resistance (Table 2), i.e., the observed $F_{2:3}$ segregation ratios were not significantly different from that expected for a single gene segregating as 1 resistant: 2 heterozygous or heterogeneous: 1 susceptible, where the heterogeneous (segregating) $F_{2:3}$ rows were derived from presumptively heterozygous F_2 plants. Aphid-resistance ratings from $F_{2:3}$ rows were continuously distributed but skewed toward resistance (Figure 4).

Individual F_3 plant aphid ratings in the SD1111RR × PI71506 $F_{2:3}$ populations were also continuously distributed, rather than bimodal, though they

TABLE 1 Chi-square Analysis of F_2 Populations in the Greenhouse Trial

Cross	F2 population	† Observed R	S	Expected (3:1) R	S	χ^2	P-value
SD1111RR × PI71506	95–1	25	6	23.25	7.75	0.53	0.47
	95–2	15	1	12	4	3.00	0.08
	95–4	38	12	37.5	12.5	0.03	0.87
	95–6	10	0	7.5	2.5	3.33	0.07
	Pooled	88	19	80.25	26.75	2.99	0.08
SD01-3219R × PI 71506	88–1	17	11	21	7	3.05	0.08
	88–2	22	4	19.5	6.5	1.28	0.26
	Pooled	39	15	40.5	13.5	0.22	0.64
	Total	161					
SD1111RR × Dowling	92–1	18	5	17.25	5.75	0.13	0.72
	92–4	12	6	13.5	4.5	0.67	0.41
	92–5	22	3	18.75	6.25	2.25	0.13
	92–6	25	8	24.75	8.25	0.01	0.92
	Pooled	77	22	74.25	24.75	0.41	0.52
SD01-3219R × Dowling	91–1	20	11	23.25	7.75	1.82	0.18
	91–3	15	11	19.5	6.5	4.15	0.04*
	91–4	19	16	26.25	8.75	8.01	<0.001**
	91–5	13	7	15	5	1.07	0.30
	95–7	11	7	13.5	4.5	1.85	0.17
	Pooled	78	52	97.5	32.5	15.6	<0.001**
	Total	229					

*, **Significant at 0.05 and 0.01 probability levels, respectively.

† **R** represents resistant individuals; **S** represents susceptible individuals. Individuals were scored as susceptible if they produced one or more aphid nymphs over the seven-day test.

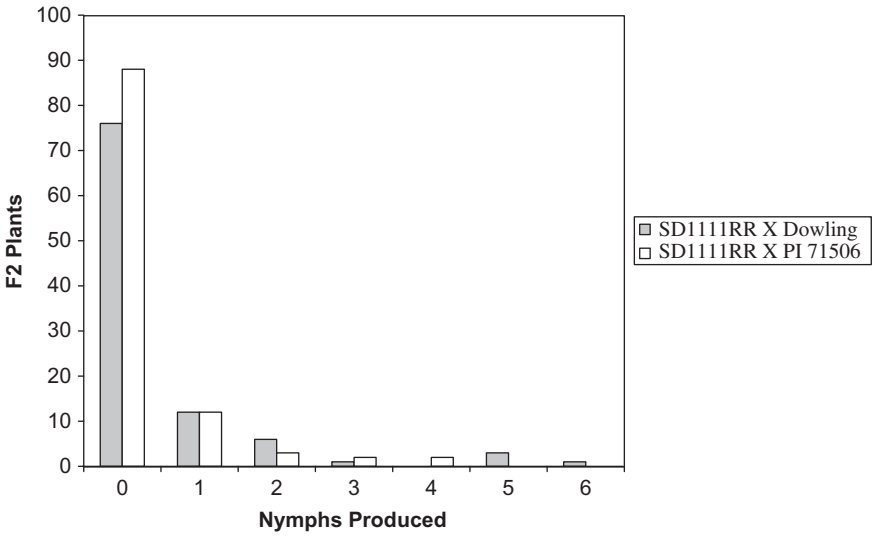

FIGURE 1 Distribution of nymph production in greenhouse F_2 populations.

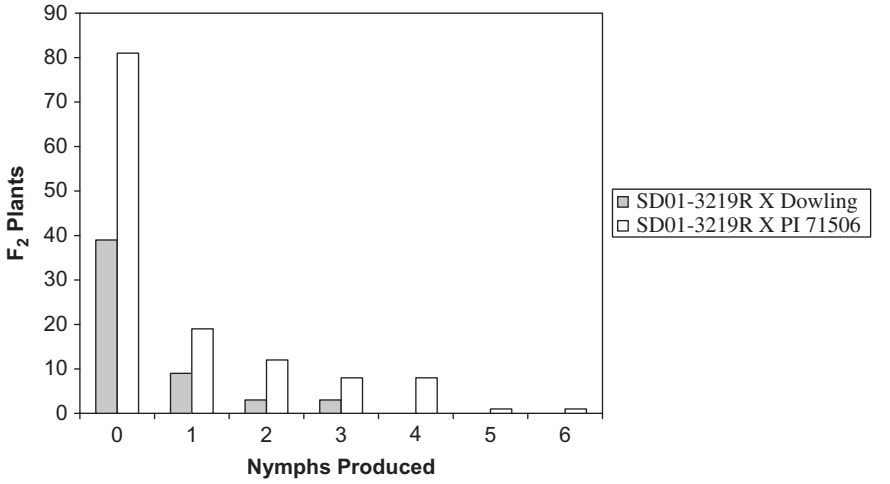

FIGURE 2 Distribution of nymph production in greenhouse F_2 populations.

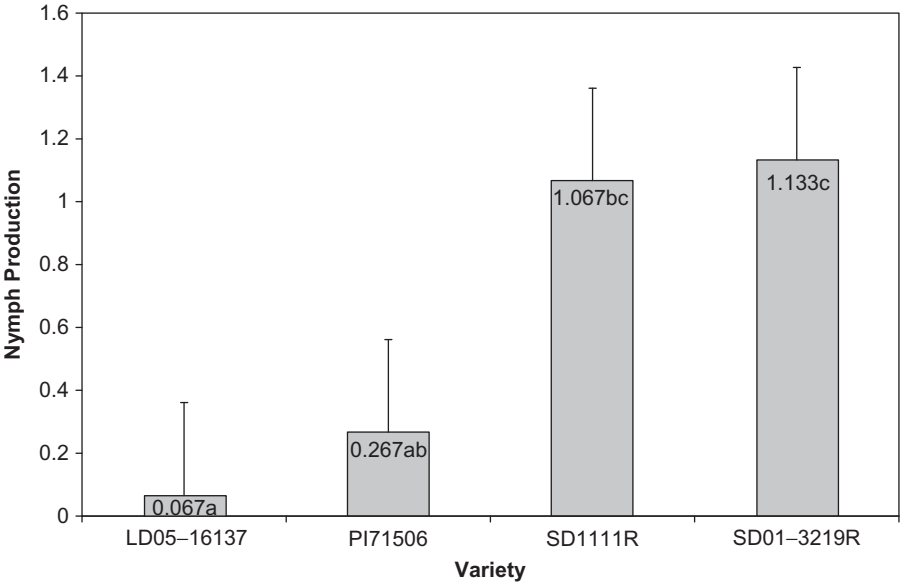

FIGURE 3 Mean nymph production on check varieties in greenhouse trial.
† Means followed by different letters are significantly different at the 0.05 significance level.

were skewed to the resistant end of the scale. Of the 555 individual $F_{2:3}$ plants evaluated, 135 plants, i.e., 24% of the population, had an aphid rating of 3, as expected in an F_3 population in which heterozygous plants display partial resistance/susceptibility associated with incomplete dominance. However, there were more resistant and fewer susceptible plants than

TABLE 2 Chi-square Analysis of $F_{2:3}$ Populations in Aphid Field-cage Study

Cross	$F_{2:3}$ population	† Observed			Expected (1:2:1)			χ^2	P-value
		R	H	S	R	H	S		
SD1111RR	95–1	9	16	2	6.75	13.5	6.75	4.56	0.10
× PI 71506	95–2	5	6	2	3.25	6.5	3.25	1.46	0.48
	95–4	16	22	14	13	26	13	1.38	0.50
	95–6	2	8	1	2.75	5.5	2.75	2.45	0.29
	Pooled	32	52	19	25.75	51.5	25.75	3.29	0.19
	Total	103							
SD1111RR	92–1	5	5	3	3.25	6.5	3.25	1.31	0.52
× Dowling	92–4	4	5	0	2.25	4.5	2.25	3.66	0.16
	92–5	9	9	1	4.75	9.5	4.75	6.79	0.03*
	92–6	17	8	3	7	14	7	19.14	< 0.001**
	Pooled	35	27	7	17.25	34.5	17.25	25.99	< 0.001**
	Total	69							

*, **Significant at 0.05 and 0.01 probability levels, respectively.

† **R** represents resistant rows (ratings 1–2); **S** represents susceptible rows (ratings 3–6); and **H** represents heterozygous or heterogeneous rows (containing both R and S rated individuals, derived from putatively heterozygous F2 plants).

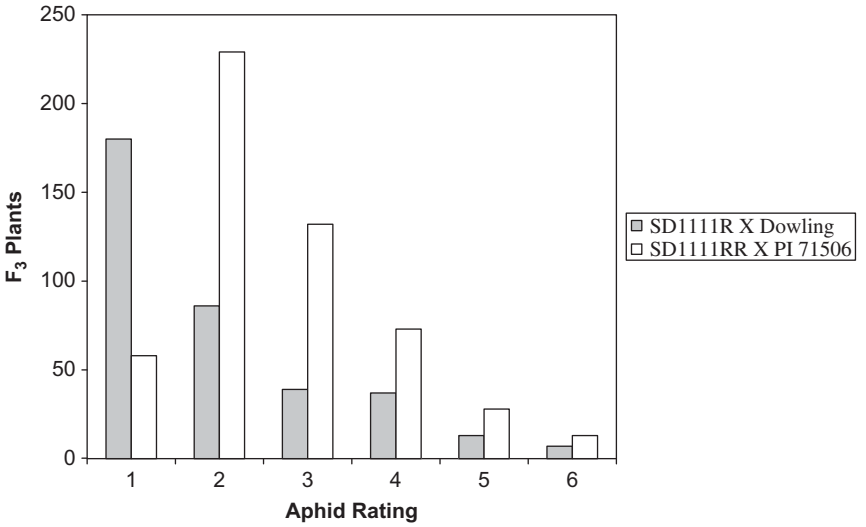

FIGURE 4 Distribution of aphid ratings of rows in $F_{2:3}$ populations.

expected, and F_3 segregation for aphid resistance did not fit a single-gene incomplete dominance model (X^2 for 3:2:3 = 63.1, $p < 0.001$).

In crosses of SD1111RR × Dowling, two of the four $F_{2:3}$ populations exhibited segregation ratios that fit a 1:2:1 single dominant-gene model (Table 2). The distribution was skewed toward resistance (Figure 4). In analyses of individual F_3 plants, only 37 individual plants out of 304 (12%) had intermediate aphid resistance ratings of 3.

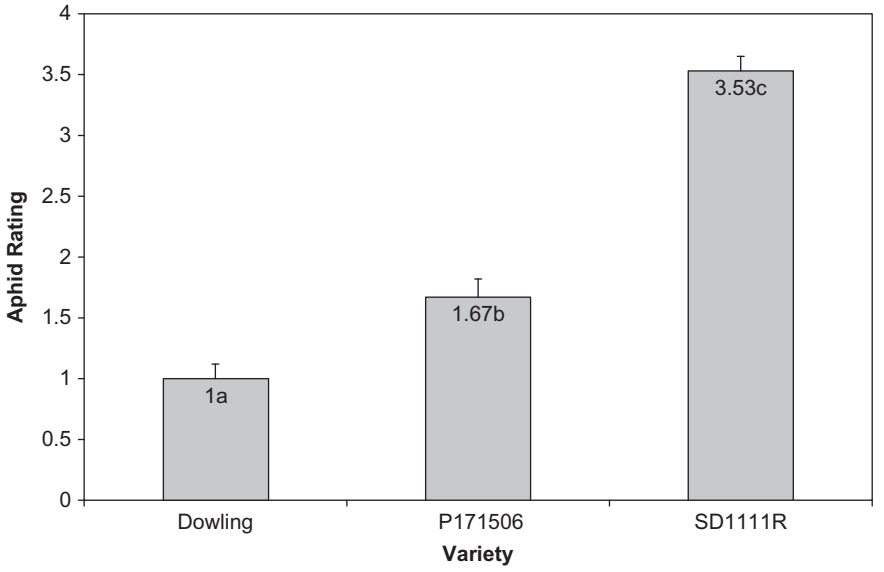

FIGURE 5 Aphid rating means of check varieties in field cage trial.
† Means followed by different letters are significantly different at the 0.05 significance level.

The frequency distributions of aphid resistance differed between the PI 71506-derived and the Dowling-derived $F_{2:3}$ population rows (Figure 4). In the Dowling-derived $F_{2:3}$ populations, the highest frequency was observed at an aphid rating of 1, corresponding to the highest level of resistance (Figure 4). In populations derived from PI 71506, the highest frequencies were at an aphid rating of 2 (Figure 4). Mean aphid numbers were also lower on Dowling check plants than on PI 71506, although both Dowling and PI 71506 had fewer aphids than the susceptible parent (Figure 5).

In the field trial of 2009, the aphid ratings of the SD1111R × PI 71506 $F_{2:5}$ population rows segregated 1:2:1 for resistant: heterogeneous or intermediate: susceptible ($X^2 = 0.325$, $p > 0.50$). Of the heterogeneous/intermediate rows, 47 (68% of the total population) were segregating for resistance/susceptibility, and 13 (16% of the total population) were homogeneously rated 3, i.e., intermediate. The parents were significantly different with mean aphid ratings of 4.5 and 1.5 for SD1111RR and PI 71506, respectively. The distribution of $F_{2:5}$ row ratings was similar to the $F_{2:3}$ distribution and skewed toward the resistant end of the scale (Figure 6).

The SD1111R × PI 71506 $F_{2:5}$ aphid ratings were significantly correlated ($r = 0.46$, $p < 0.0001$) with the aphid ratings of the individual $F_{2:3}$ plants from which they were derived. When individual families, however, were examined, the relationship held only within one family (95–4). Within this family, regression of $F_{2:5}$ mean row rating on $F_{2:3}$ plant rating was highly

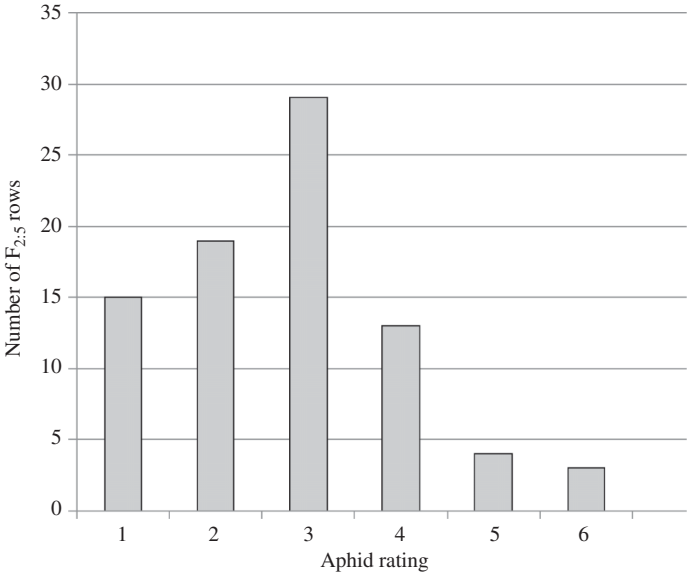

FIGURE 6 Distribution of aphid ratings in SD1111RR × PI 71506 $F_{2:5}$ population rows.

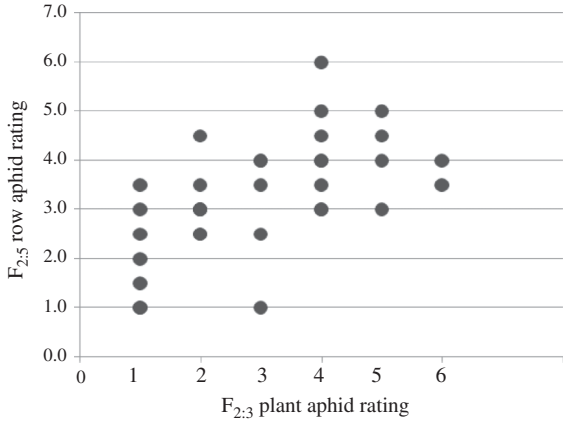

FIGURE 7 Segregation of SD1111RR × PI 71506 family 95–4 $F_{2:5}$ row aphid ratings in relation to the individual $F_{2:3}$ parent plants from which they were derived.

significant ($p < 0.0001$), with $R^2 = 0.28$. Figure 7 shows the relation of $F_{2:5}$ row ratings to the aphid rating of their $F_{2:3}$ parents. None of the $F_{2:3}$ plants that had been rated 4 and above produced any aphid-resistant (rating of 1–2) progeny in the $F_{2:5}$ generation. However, $F_{2:3}$ plants rated 1, 2, or 3 produced segregating $F_{2:5}$ progeny, including aphid-resistant (rated 1–2) plants.

In the greenhouse trial using the Ohio isolate, aphid population means differed significantly among varieties (Figure 8). PI 71506 had significantly

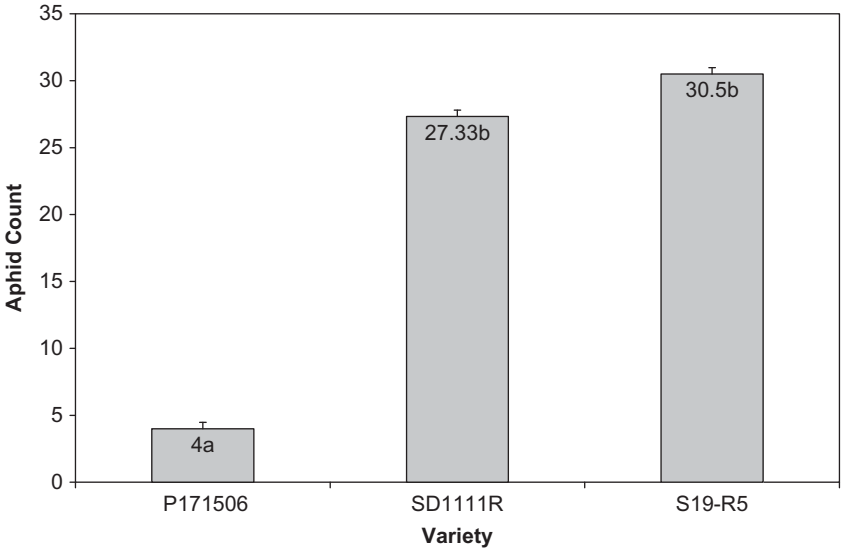

FIGURE 8 Ohio aphid biotype count means of check varieties in greenhouse trial.

† Means followed by different letters are significantly different at the 0.05 significance level.

fewer aphids than the susceptible varieties SD1111RR and S19-R5 in this choice test.

DISCUSSION

Nymph production in the no-choice greenhouse trial segregated as expected assuming aphid resistance was conferred by a single dominant gene from PI 71506. Segregation of aphid resistance in all of the SD1111R × Dowling populations and two of the SD01-3219R × Dowling populations confirmed the expected model of resistance associated with the single dominant gene *Rag1* (Hill, Li, & Hartman 2006). However, two F_2 populations of the SD01-3219R × Dowling cross did not fit the expected 3:1 segregation ratio.

Population distributions for nymph production were skewed toward lower levels of nymphs (Figures 1 and 2), as expected if resistance was attributable to a single dominant gene, and this was observed for both the PI 71605 and the Dowling crosses to either susceptible parent. However, the low and variable rate of nymph production may have biased resistance scores toward an overestimation of the number of resistant plants, and compromised the ability of the assay to discriminate between PI 71506 and SD1111RR. Any antixenotic components of PI 71506 resistance would not have been measured by this no-choice assay.

Field cage screening of $F_{2:3}$ lines from SD1111RR × PI 71506 suggested that resistance from PI 71506 was controlled by a single gene in all four populations (Table 2). However, the distribution of resistance was

continuous (Figure 4) rather than bimodal, indicating intermediate levels of resistance/susceptibility and suggesting incomplete dominance effects or possible contributions from additional genes. Individual plant aphid ratings in the SD1111RR × PI 71506 $F_{2:3}$ populations were also continuously distributed rather than bimodal, though skewed to the resistant end of the scale. A continuous distribution would be expected in an F_3 population in which heterozygous plants display partial resistance/susceptibility associated with incomplete dominance. A bimodal distribution is expected for resistance associated with a single dominant gene, e.g., in Dowling-derived populations segregating for *Rag1* resistance (Hill, Li, & Hartman 2004). Thus, PI 71506-derived resistance may not be completely dominant. The skewing of frequencies toward more resistant plants also suggested that more than one gene may contribute to resistance from PI 71506.

Two of the Dowling-derived $F_{2:3}$ populations segregated as expected, but two had more resistant rows than expected and did not fit the single-gene model. This finding may be partly explained by the rating scale, which did not account for the differences in resistance level that existed between Dowling and PI 71506. While it may have accurately categorized resistance within populations derived from PI 71506 crosses, it may have been inaccurate in categorizing populations formed from Dowling crosses (Table 1, Figure 5). This was particularly true when determining which SD1111RR × Dowling $F_{2:3}$ rows resulted from a heterozygous parent. If the break between resistance and susceptibility was set too high, resistant plants were overestimated; conversely, if set too low, susceptible plants were overestimated. In this experiment, the rating was set too high for the Dowling × SD1111RR $F_{2:3}$ populations and the resistant rows were overestimated. When resistant and heterozygous plants were combined and tested against a 3:1 dominant-gene model, the 92–6 population fit the model but the 92–5 population did not ($p = 0.046$).

Although the resistance ratings used in this experiment did not always separate resistant and heterozygous rows in SD1111RR × Dowling populations, segregation of *Rag1* resistance did match a 3:1 single dominant gene model in three out of four populations. Furthermore, when the Dowling-derived populations were rated on a scale where only aphid ratings of 1 were considered resistant, the segregation of the pooled population data did not deviate significantly from a single dominant-gene model for resistance ($X^2 = 2.99$, $p > 0.10$). It should be noted, however, that *Rag1* resistance does not always appear to be completely dominant in all populations (Carter et al. 2007), and it is possible that in some Dowling-derived crosses *Rag1* heterozygotes may have lower levels of resistance than homozygous plants.

The level of aphid resistance in PI 71506 was slightly lower than that of Dowling in the aphid field-cage trial (Figure 5), and this was also expressed in the different distributions of the segregating $F_{2:3}$ populations (Figure 4).

The highest frequency in the PI 71605-derived populations centered around a rating of 2, whereas in the Dowling-derived populations the highest frequency was around an aphid rating of 1. The differences in resistance between PI 71506 and Dowling may be due to the type or strength of resistance they expressed. Hill, Li, and Hartman (2004) and Hesler, Dashiell, and Lundgren (2007) both suggested that PI 71506 primarily displayed antixenosis or non-preference resistance. Hesler, Dashiell, and Lundgren (2007) further characterized resistance in PI 71506 and found that it displayed some antibiosis qualities, although not as strong as the antibiosis resistance in Dowling. These differences in resistance type may have directly affected how resistance was expressed in the aphid-cage populations. Differences in level, expression, and segregation of resistance indicated that a different allele at the *Rag1* locus, or a gene other than *Rag1*, may control or contribute to resistance in PI 71506 as suggested by aphid resistance in three-way cross populations derived from both PI 71506 and Dowling (Carter et al. 2007). The apparent genomic differences between PI 71506 and Dowling, as described by Chen et al. (2007), also suggest that differences in the nature and genetics of aphid resistance between PI 71506 and Dowling or other sources of resistance would not be unexpected.

In the field trial of 2009, the aphid ratings of the SD1111RR × Dowling $F_{2:5}$ rows segregated 1:2:1 for resistant: heterogeneous/intermediate: susceptible. The rating scale used for measuring resistance in this assay was somewhat more stringent, but corresponded to the performance of the parent lines for resistance/susceptibility, and allowed us to distinguish potential incomplete dominance effects associated with heterozygosity in addition to heterogeneity of segregating rows. The distribution (Figure 6) was similar to that of the $F_{2:3}$ population, indicating single-gene segregation skewed toward resistance, suggesting dominance effects. However, the persistence of intermediate ratings suggested that if PI 71506 resistance was due to a single gene, the PI 71506 allele may not be completely dominant, and additional PI 71506 genes may also contribute to resistance. Regression of $F_{2:5}$ row aphid ratings on the aphid ratings of the individual $F_{2:3}$ plants from which they were derived (Figure 7) showed segregation for resistance in the progeny of resistant and intermediate $F_{2:3}$ plants, but none from susceptible $F_{2:3}$ plants, demonstrating that susceptibility appeared to be associated with recessive allele(s).

The discovery of new aphid biotypes provides another way to characterize aphid resistance in soybean accessions (Kim et al. 2008). The Ohio aphid isolate is one biotype that has been shown to overcome *Rag1* resistance (Kim et al. 2008). We used the Ohio aphid isolate to further characterize the resistance associated with PI71506 and to determine if the Ohio isolate could overcome the resistance gene in PI71506. Our analysis showed that the resistance conferred by PI 71506 was effective against the Ohio isolate.

Further choice and no-choice testing of PI 71506 in direct comparison with Dowling are needed to assess possible differences.

CONCLUSIONS

In summary, soybean aphid resistance in PI 71506 fit a single-gene model in three generations of aphid screening, showing primarily dominance effects, although additional genes may also contribute to resistance from PI 71506. Comparisons with *Rag1* resistance from Dowling confirmed these findings and also demonstrated differences between PI 71506 and Dowling in the expression and segregation of resistance. The resistance of PI 71506 to aphids was somewhat weaker than that of Dowling; however, PI 71506 maintained antixenosis resistance to an Ohio aphid isolate that has overcome *Rag1* resistance.

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